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Pyranocoumarins: A new class of anti-hyperglycemic and anti-dyslipidemic agents

Atul Kumar [a,*], Ram Awatar Maurya [a], Siddharth Sharma [a], Pervez Ahmad [a], A. B. Singh [b], Gitika Bhatia [b], Arvind K. Srivastava [b]

[a] Medicinal and Process Chemistry Division, Central Drug Research Institute, Council of Scientific & Industrial Research, Lucknow 226 001, India
[b] Biochemistry Division, Central Drug Research Institute, Council of Scientific & Industrial Research, Lucknow 226 001, India

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ABSTRACT

A series of pyranocoumarin derivatives were synthesized and evaluated in vivo for their anti-hyperglycemic as well as anti-dyslipidemic activities. Compounds **7a**, **7c**, **8a**, **8b**, **8c**, **8e** and **8f** have shown promising anti-hyperglycemic activities in sucrose loaded model (SLM) as well as sucrose challenged streptozotocin induced diabetic rat model (STZ). Compounds **8a** and **8b** were showing 38.0% and 42.0% blood glucose lowering activity in db/db mice model. In vitro anti-hyperglycemic activity evaluation exhibited that compounds **8a** (IC_{50} = 24.5 μM) and **8b** (IC_{50} = 36.2 μM) are potential PTP-1B inhibitors thereby revealing their possible mechanism of anti-diabetic action. Compounds **7a**, **7b**, **8a**, **8b**, **8d**, **8e** and **8f** have shown significant anti-dyslipidemic activity in triton induced dyslipidemia in rats.

Diabetes mellitus is multifactorial disease characterized by high level of blood glucose and impaired insulin action.[1] It is an independent risk factor for the development of coronary artery diseases, myocardial infarction, hypertension, and dyslipidemia. Non-insulin dependent diabetes mellitus (NIDDM) accounts for approximately 80–90% of all diabetes cases.[2] The number of diabetic complains are continuously growing with a currently estimated worldwide incidence of about 194 million people and expected to increase to 330 million by 2025.[3] When the carbohydrates are not present in sufficient amount or their metabolism is impaired, fats become the principle source of energy. Fatty acids are mobilized into the general circulation leading to secondary triglyceridemia in which total serum lipids in particular triglycerides as well as the levels of cholesterol and phospholipids increases. This rise is proportional to the severity of the diabetes. Uncontrolled diabetes is manifested by a very high rise in triglycerides and fatty acid levels.[4] An increase in plasma lipids, particularly cholesterol, is a common feature of atherosclerosis, a condition involving arterial damage, which may lead to ischemic heart disease, myocardial infarction, and cerebro-vascular accidents. These conditions are responsible for one-third of deaths in industrialized nations.[5] Therefore, an anti-hyperglycemic drug, having lipids (triglycerides and cholesterol) lowering activity is of great demand. Several research groups have focused their attention to develop such dual-acting agents.[6] Herein, we describe design, synthesis, anti-hyperglycemic and anti-dyslipidemic activity of pyranocoumarin derivatives. The design of pyaranocoumarin is based on natural products exhibiting promising antidiabetic activity such as Sanggenone C,[7] Luvangetin,[8] and Rutamarin.[9] These natural products have coumarin as central nucleus along with pyran or furan scaffolds present in linear arrangements. Based on this we have designed and synthesized pyranocoumarin having angular arrangements for their anti-hyperglycemic and lipid lowering activity (Fig. 1).

The key intermediate 8-acetyl-7-hydroxy-4-methyl-2*H*-chromen-2-one **5** was synthesized from resorcinol **1** in four steps. Resorcinol **1** was treated with ethyl acetoacetate **2** and concentrated H_2SO_4 to yield 7-hydroxy-4-methyl-2*H*-chromen-2-one **3**. 7-Hydroxy-4-methyl-2*H*-chromen-2-one **3** on reaction with acetic anhydride in pyridine gave 4-methyl-2-oxo-2*H*-chromen-7-yl acetate **4**. Fries migration of the acetyl group of compound **4** by $AlCl_3$ at 170 °C resulted to the formation of 8-acetyl-7-hydroxy-4-methyl-2*H*-chromen-2-one **5**. Compound **5** was treated with paraformaldehyde and amine in different optimized reaction conditions resulting to the formation of a range of pyranocoumarins. 8-Acetyl-7-hydroxy-4-methyl-2*H*-chromen-2-one **5**, when refluxed with 2 equiv of paraformaldehyde in ethanol afforded 9-ethoxymethyl-4-methyl-8, 9-dihydro-pyrano[2,3-*f*]chromene-2,10-dione **6** in 62% yield. Refluxing 8-acetyl-7-hydroxy-4-methyl-2*H*-chromen-2-one **5** with 3 equiv of paraformaldehyde and 1 equiv of amine in ethanol afforded compounds **7a–c** and **8a–h**. 8-Acetyl-7-hydroxy-4-methyl-2*H*-chromen-2-one **5**, when refluxed with 3 equiv of paraformaldehyde and 2 equiv of amine in ethanol afforded compounds **9a–h** in

* Corresponding author.
E-mail address: dratulsax@gmail.com (A. Kumar).

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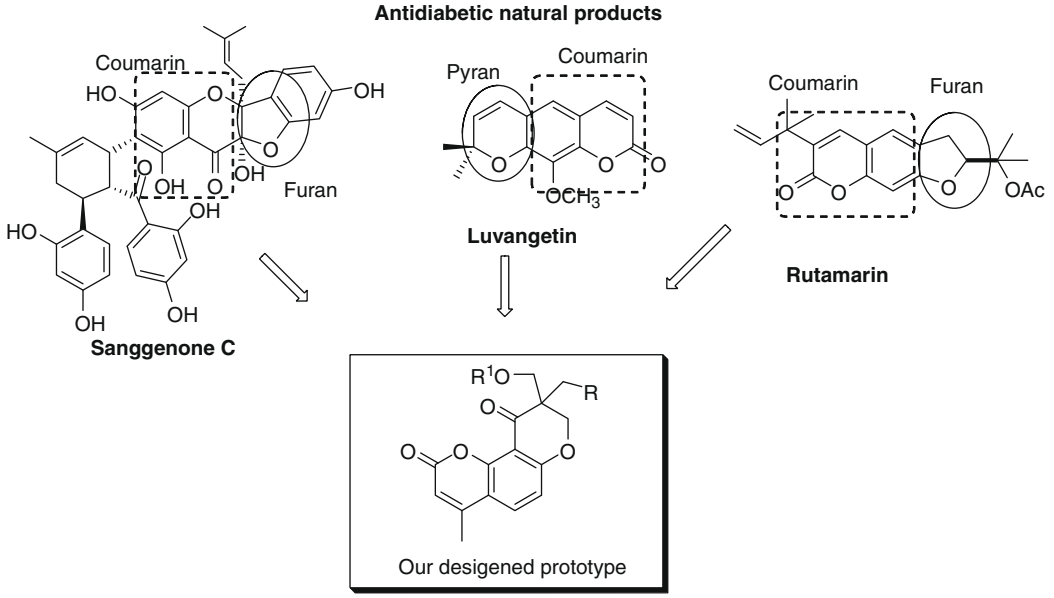
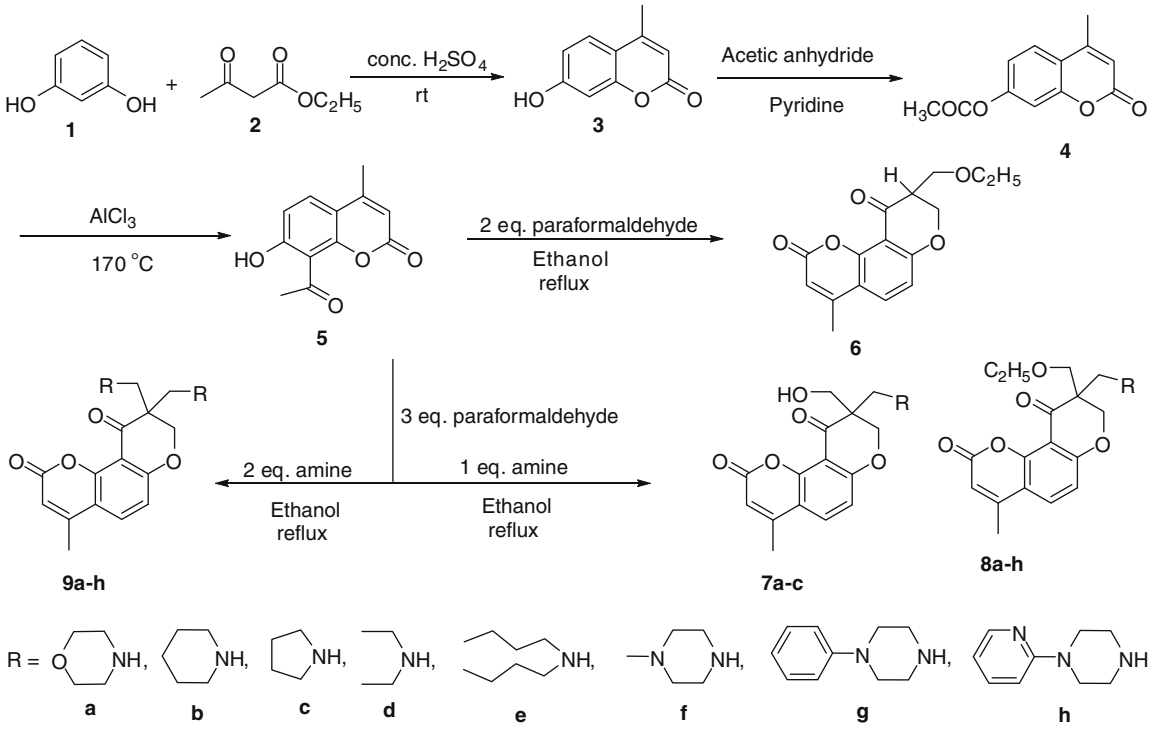


Figure 1. Designing of novel pyranocoumarins based on natural antidiabetic scaffolds.

60–75% yields. The systematic representation of pyranocoumarin synthesis is shown in Scheme 1.

Initially the synthesized compounds **7a–c**, **8a–h** and **9a–h** were screened in vivo for their anti-hyperglycemic activity in sucrose loaded model (SLM). Metformin was taken as standard drug in SLM model which showed 25.0% blood glucose lowering activity at the dose of 100 mg/kg. In SLM model compounds **7a** (28.2%), **7c** (30.3%), **8a** (35.5%), **8b** (38.1%), **8c** (31.8%), **8e** (30.6%) and **8f** (32.5%) showed blood glucose lowering activity respectively at a dose of 100 mg/kg body weight (Fig. 2). It was interesting to note that compound with diamine side chain (**9a–h**) showed poor anti-hyperglycemic activity but with mono amine side chains (**7a–c** and **8a–h**) were showing promising anti-hyperglycemic activity.

All the compounds which exhibited greater anti-hyperglycemic activity than metformin in SLM model were further screened in sucrose challenged streptozotocin induced diabetic rat model (STZ). In STZ model, compounds **7a** (25.3%), **7c** (24.0%), **8a** (35.5%), **8b** (37.7%), **8c** (28.8%), **8e** (24.4%) and **8f** (18.7%) showed blood glucose lowering activity respectively at a dose of 100 mg/kg body weight after 24 h treatment whereas the standard drug metformin showed 26.6% anti-hyperglycemic activity (Fig. 3).



Scheme 1. Synthesis of novel pyranocoumarins.

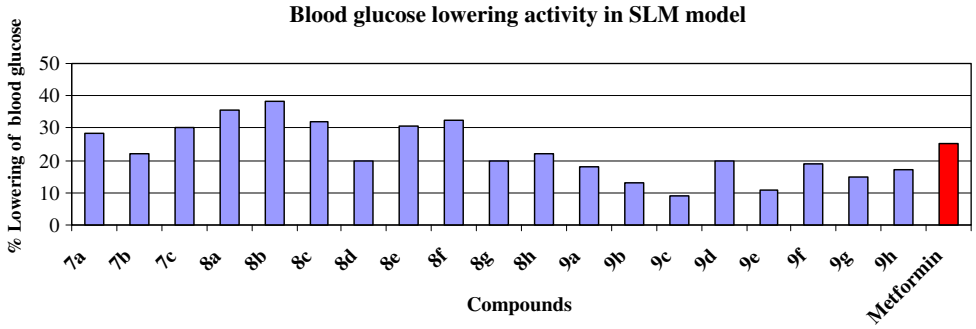


Figure 2. Anti-hyperglycemic activities of compounds **7a–c**, **8a–h**, and **9a–h** in SLM.

Two of the most active compounds in STZ model (**8a** and **8b**) were further screened for their anti-hyperglycemic activity in db/db mice which is a well-characterized model of type 2 diabetes. The background for the db/db mouse is the C57BL/Ks strain. The major deficiency of the C57BL/KsBom-db mouse (db/db mouse) is lack of a functional leptin receptor. This leads to defective leptin signaling and a complete lack of feedback from leptin. Both hypothalamic NPY content and secretion are consequently elevated, and this result in hyperphagia and decreased energy expenditure, obesity, insulin-resistance, hyperinsulinemia, hyperglycemia and dyslipidemia. The optimal age of db/db mice used for experiments is from week 12 to 18 when they have developed NIDDM with diabetic dyslipidemia but still have functional β-cells in the pancreas. The two compounds exhibited 21.1% (**8a**), 19.5% (**8b**) after 5 days and 38.0% (**8a**), 42.0% (**8b**) blood glucose lowering activity after 10 days treatment respectively. The standard drug rosiglitazone showed 22.0% after 5 days and 48.1% blood glucose lowering activity after 10 day treatment in similar conditions at a dose of 100 mg/kg (Fig. 4).

In order to study the possible mechanism of anti-hyperglycemic activity of these compounds (**7a–c, 8a–f** and **9a–f**), we evaluated them for their in vitro anti-hyperglycemic activity against the glucose-6-phosphatase, glycogen phosphorylase and α-glucosidase, DPP IV and PTP-1B enzymes at 100 μM concentrations (Table 1).

The effect of compounds on glucose-6-phosphatase was studied by pre-incubating the compound in 1.0 mL reaction system for 10 min and then determining the residual glucose-6-phosphatase activity according to the method of Hubscher and West.[10] The glycogen phosphorylase activity is measured by the modified method of Rall et al.[11] Effect of compounds on α-glucosidase was determined by method of Lebovitz[12] The dipeptidyl peptidase enzyme inhibition assays were performed according to the methods of Wright et al.[13] Protein Tyrosine Phosphatase 1B activity was determined by the modified method[14] using *p*NPP as substrate.

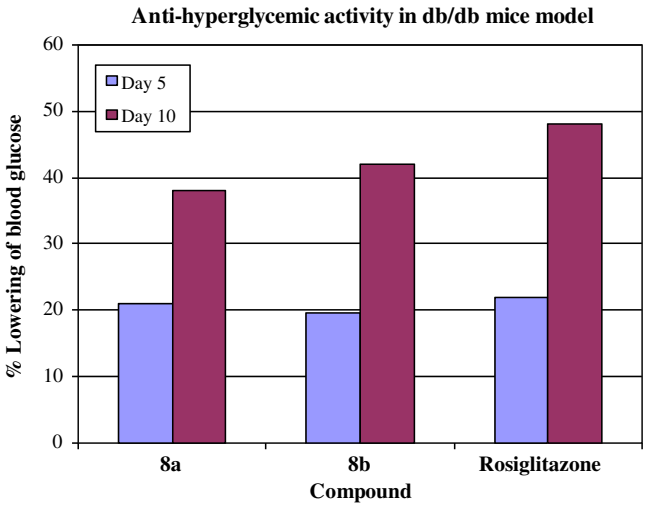


Figure 4. Anti-hyperglycemic activities of compounds **8a**, **8b** and rosiglitazone in db/db mice model.

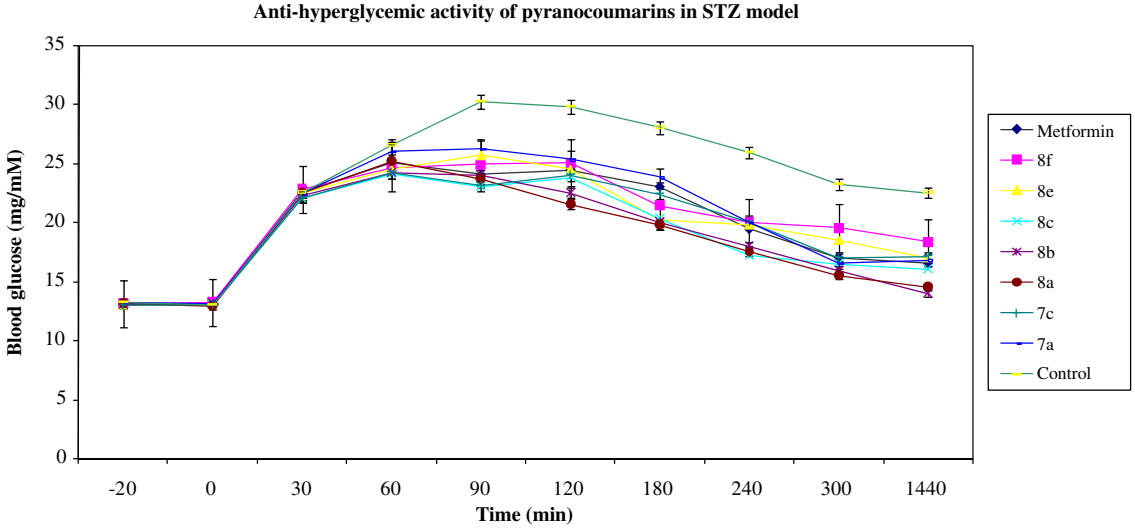


Figure 3. Anti-hyperglycemic activities of compounds **7a**, **7c**, **8a**, **8b**, **8c**, **8e**, and **8f** in STZ model.

Table 1
In vitro anti-hyperglycemic activity of compounds **7a–c**, **8a–h** and **9a–h**

Compounds	% Inhibition[a]				
	Glucose-6-phosphatase	Glycogen phosphorylase	α-Glucosidase	DPP IV	PTP 1B[b]
7a	3.50	NI	34.3	12.0	83.3 (52.4)
7b	7.36	NI	37.3	NI	64.0 (62.1)
7c	5.83	8.3	22.1	8.3	74.6 (56.7)
8a	12.45	8.4	NI	NI	92.2 (24.5)
8b	6.56	8.5	NI	12.6	88.5 (36.2)
8c	8.31	8.3	12.0	13.2	72.1 (82.7)
8d	24.23	NI	NI	NI	74.1 (90.4)
8e	11.10	8.7	27.9	NI	80.5 (40.5)
8f	17.43	26.9	NI	7.9	70.3 (65.5)
8g	31.67	NI	16.2	NI	62.3 (70.6)
8h	9.67	30.1	11.3	NI	54.5 (76.2)
9a	6.78	12.8	8.3	8.8	24.68
9b	13.8	17.2	7.3	6.8	33.27
9c	8.28	15.2	NI	8.2	29.78
9d	9.45	12.2	8.5	8.1	41.34
9e	20.39	11.1	NI	11.4	43.62
9f	12.96	10.2	6.4	12.5	21.98
9g	21.54	22.1	15.9	15.2	28.67
9h	28.00	11.0	15.8	9.8	41.34
Sod. *ortho*-vanadate		–	–	–	57.8

[a] Compounds were evaluated at 100 μM concentration; NI means no inhibition.
[b] IC_{50} (μM) values are given in parentheses.

Although the compounds (**7a–c**, **8a–h**, **9a–h**) were showing poor inhibitory activities for glucose-6-phosphatase, glycogen phosphorylase, α-glucosidase and DPP IV enzymes, compounds (**7a–c, 8a–h**) have shown promising PTP 1B inhibitory activity. It was interesting to note that compounds with diamine side chain (**9a–h)** exhibited moderate whereas compounds with monoamine side chain (**7a–c**, **8a–h**) exhibited high PTP 1B inhibitory activity. From these observations, we concluded that PTP 1B might be a possible target for these anti-hyperglycemic compounds.

The anti-dyslipidemic activities of compounds **7a–c, 8a–h** and **9a–h** were evaluated in a triton model.[15] Administration of triton WR-1339 in rats (Charles Foster strain, male, adult, body weight 200–225 g) induced marked hyperlipidemia as evidenced by increase in the plasma levels of total cholesterol (TC) (3.50 F), phospholipids (PL) (3.12 F), triglyceride (Tg) (3.14 F). Treatment of hyperlipidemic rats with compounds (**7a–c, 8a–h** and **9a–h**) at dose of 100 mg/kg po reversed the plasma level of lipids with varying extents. Compounds **7a**, **7b**, **8a**, **8b**, **8d**, **8e** and **8f** showed significant lipid lowering activity in plasma level of TC, PL and Tg respectively, while other compounds showed mild lipid lowering activity as compared to triton. These data were compared with Gemfibrozil at a dose of 100 mg/kg, which showed a decrease in plasma levels of TC, PL and Tg by 37.1%, 39.1% and 36.1%, respectively at the dose of 100 mg/kg (Fig. 5).

Structural activity relationship reveals that compounds having ether group were showing better antidiabetic activity than corresponding hydroxyl group. Nitrogen containing six member rings (**8a** and **8b**) were exhibiting more potent antidiabetic activity in vivo as well as PTP-1B inhibition in comparison to five member and open chain nitrogen containing compounds. Compound **8b** having piperidine moiety was most potent compound of the series. It is interesting to note that introduction of nitrogen in piperidine ring (**8f**, **8g** and **8h**) resulted in loss of antidiabetic and PTP 1B inhibitory activity however incorporation of oxygen in piperidine ring (**8a**) have shown similar activity profile as of **8b**.

In conclusion, we have synthesized a series of pyranocoumarins derivatives (**7a–c**, **8a–h**, **9a–h**) and evaluated their anti-hyperglycemic as well as anti-dyslipidemic activities. Several compounds of the series have shown promising anti-hyperglycemic activity in SLM, STZ and db/db mice models. Some compounds of the series (**8a** and **8b**) were found potential anti-hyperglycemic agents comparable to standard drugs. In vitro anti-hyperglycemic activity evaluation revealed that compounds **8a** (IC_{50} = 24.5 μM) and **8b** (IC_{50} = 36.2 μM) are potential PTP 1B inhibitors thereby revealing

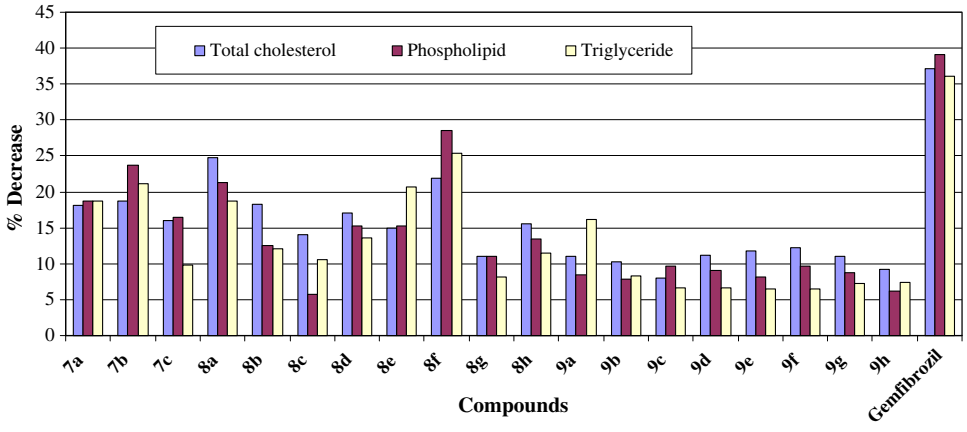


Figure 5. Anti-dyslipidemic activities of compounds **7a–c**, **8a–h** and **9a–h**.

their possible mechanism of anti-diabetic action. Compounds **7a**, **7b**, **8a**, **8b**, **8d**, **8e** and **8f** were showing significant anti-dyslipidemic activity in triton induced rat model.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.09.031.

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